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ON GERMINAL TRANSPLANTATION IN VERTEBRATES.

1. INTRODUCTION.

The scientific results described in this paper were obtained from experiments begun in the Zoölogical Laboratory of Harvard University and completed in the Laboratory of Genetics of the Bussey Institution. These experiments were made possible by a grant from the Carnegie Institution of Washington to the senior author, for which grateful acknowledgment is hereby made. The authors desire also to thank Dr. Alexis Carrel, of the Rockefeller Institute, for valuable suggestions as to operative technique.

The curiosity of zoologists has long been aroused to know whether the reproductive gland of a vertebrate can be successfully transplanted from the body of one individual to another; and, if so, whether the gland will thereafter function in its new environment; and, if it does, whether the nature of its products will remain unaltered. The fact has repeatedly been pointed out that experiments of this sort, if successful, should afford a crucial test of the Lamarckian and the Weismannian views, respectively, of the relation of the germinal substance to its environment and in particular to the body.

Our own attention was particularly directed to these questions by the remarkable results recently described by Guthrie and Magnus, which seemed to show that transplanted ovaries, in a foreign body, liberate products distinctly influenced in nature by that body. To test the correctness of such a conclusion the experiments described in this paper were undertaken. Since it is known that the environment directly influences the nature of the body, if it can be shown further that the body directly influences the character of the inheritance through the sexual products, the Lamarckian principle is established and that of Weismann is disproved. It is therefore of fundamental importance either to confirm or to disprove the results of the authors mentioned.

We are unable to confirm, we present evidence which tends to disprove, the conclusions reached by Guthrie and Magnus. We do not question the results reported by them, but only the interpretations given by them to that work.

Every biologist is familiar with the able series of essays in which Weismann showed the physiological distinctness of body and germ-plasm. Many will recall also the noteworthy experiments of Heape (1890–1897), by which he showed that influences exerted during gestation do not modify the inher-

itance. Heape's evidence was this: The fertilized egg of a rabbit of one variety (for example a long-haired albino) was removed from the oviduct of the mother before it had become attached to the uterine wall. It was then transferred immediately to the oviduct of a rabbit of a different variety (for example a Belgian hare, which is neither white nor long-haired). In several cases the transferred egg became attached in its new position and passed through all the stages of gestation. Young rabbits produced in this way were both long-haired and albinos like the mother of the eggs, not like the rabbit which bore the young. The foster-mother, indeed, seems not to have influenced the inheritance any more than the corn supplied to cattle determines their breed characters.

Granting all this, a further question remains to be dealt with. The transferred egg was already full-grown and fertilized. If the transfer had been made at an earlier stage while the egg was still growing, would the results have been the same? Might not the *growing egg* have lacked that selective power in assimilation which belongs to the full-grown and fertilized egg? May not the former be subject to modification by the environment, even though the latter is not?

This is the question involved in a study of germinal transplantation. Guthrie believes that he has found evidence of such modification; we question the validity of this evidence, on what grounds will presently appear. On the other hand, we present one clear case of the transplantation of an undeveloped ovary, which later liberated eggs in the body of a foster-mother, but in the young so produced no foster-mother influence is detectable. We therefore question still the existence of foster-mother influence. We maintain with Weismann not only that modifications of the body are not handed on to the germ-plasm, but that the character of the body does not in the least influence the character of the contained germ-plasm, provided only the body affords a suitable medium within which the germ-plasm may exist.

2. REVIEW OF THE LITERATURE ON OVARIAN GRAFTING IN ANIMALS OTHER THAN MAN.

Beginning with the year 1895, a large number of investigators have given attention to ovarian grafting. The results have often been conflicting. They represent the work of physiologists, pathologists, biologists, experimental and clinical surgeons, and lastly of students of heredity. It is impossible here to give space for the discussion of the entire subject. All that is proposed is a brief review of its more important aspects.

As now understood, the term autoplastic grafting means the transfer of tissue within the body of the individual, while homoplastic means the transfer of tissue between individuals of the same species. A third term, heteroplastic grafting, is used to denote a transfer between two individuals of different species or genera.

It is proposed to review very briefly the work done in each of these fields.

Knauer (1896) was the first to report on a series of experiments with animals. He was led to undertake this work through Chrobak, a surgeon who had himself tried feeding ovarian substance to women in whom an artificial menapause, with its attendant train of symptoms, had been brought about by operation. Chrobak's results were not conclusive, and he thought surgical grafting held out more hope of relief in these cases.

In his several papers (1896–1900) Knauer, who worked on rabbits, showed by a series of twelve autografts that the transplanted ovary persisted in its new location even up to three years; that its appearance was normal; that genital atrophy was prevented; and further, that it was possible for animals so operated upon to bear young. He gives details of his very careful technique. The ovaries, after the castration, were placed either in the mesometrium, on the horn of the uterus, or between the fascia and muscles of the abdominal wall.

Grigoryeff (1897) confirmed Knauer's work in all its aspects and reported normal young born from his rabbits after castration and autoplastic removal of both ovaries.

Ribbert (1897–1898) made careful histological examinations of autoplastic ovaries, studying the initial process of destruction followed by reconstruction. As late as 150 days after the operation he found no atrophy.

Fish, Rubinstein, Halban, Herlitzka, Basso, Carmichael, Katsch, Stilling, Limon, and others also proved that autoplastic grafting is possible. From their work one comes to the conclusion that autografts of whole ovaries on animals should nearly always be successful, provided the technique is careful and the ovary not too large or too old. Success does not appear to depend upon the new position of the ovary. It will grow anywhere where nourishment is assured, and will even establish itself at times when merely dropped into the peritoneal cavity. In connection with this, one must remember the experiment of Lode, who injected the ova of *Ascaris* into the abdominal cavity of animals and afterwards recovered these from the fallopian tubes and uterus.

Among all these workers Arendt raises the only dissenting voice. He concludes that neither autoplastic nor homoplastic ovarian grafting is possible. He criticizes Knauer's work and the clinical work of Glass, Morris, Montprofit, and others, but his conclusions are clearly too sweeping.

We have ourselves obtained several normal young from a rabbit whose own ovaries were grafted onto the uterine horns. Both the ovaries were found large and healthy in their new position at the end of nine months.

In studying the results of homoplastic grafting we obtain, however, a very different picture and are forced to conclude that the success in this group depends not only on good technique, but also perhaps on the relationship of the two stocks, and certainly on the intimate chemical tolerance of the

opposed tissues. Thus the results are greatly at variance. No doubt the stock used by some of the workers in this field has been more or less closely related. The ratio of success in such cases to the degree of relationship of the opposing tissues has not been worked out, so far as we are aware.

It is necessary to criticize workers in this group on the ground that many cases are considered successful if the ovary is found more or less normal after short intervals—days or weeks. This is no adequate test, as in many of these cases degeneration is ultimately complete.

Knauer's results with his thirteen operations of homoplastic grafting were negative except in two cases, in which the findings were by no means conclusive. In spite of this Knauer thought that homoplastic grafts were possible, though difficult.

Fish (1899) experimented upon twenty rabbits with, he says, successful results. He had hoped to establish the fact that conception is possible subsequent to homoplastic ovarian transplantation, but in this he was disappointed. His experiment is not given in detail.

Foa (1900–1901) was led to undertake experiments in homoplastic grafting, at the suggestion of Celisea, as a means of settling the controversy between the Neo-Lamarckians and the Neo-Darwinians. He does not give details of each experiment, and their number appears to have been small. His grafts were made onto the original ovarian sites. With this method one might question whether regenerated tissue could be distinguished from grafted tissue. His conclusions were that homoplastic grafting was practical, especially when ovaries of new-born animals were used as material to be grafted. He thought that such material, planted into older animals, grew much faster than normally and soon arrived at the growth stage of the host, and he cites an experiment in support of this view. It is possible, however, that regeneration of older tissue may have occurred in such cases rather than accelerated development of introduced tissue. He says also that ovaries of a new-born animal immediately degenerate when placed in an older animal whose own ovaries have ceased to function. His findings inclined him to the belief that there can not be such independence of germinative material as the doctrine of Weismann would have us believe. Experiments in which he hoped to show the influence of the foster-mother upon foreign germinative material have not been heard from.

Guthrie (1908) did homoplastic grafting of ovaries in chickens and obtained young from his grafted animals. He concludes that the homoplastic ovaries function normally and produce young. He thinks that the color characteristics of the fœtuses and of the chicks may be influenced by the foster-mother. The detailed observations made may be summarized as follows:

Two pure-bred black single-comb Leghorn and two white single-comb Leghorn pullets were operated upon, and a third pullet of each sort was

kept as control. In the operations the ovary of a black pullet was exchanged with that of a white one. Six months after the operation the birds, which had now begun to lay, were mated, with the following results in chicks or fœtuses:

1. The control black hen mated with a black cock produced thirteen black chicks with light breasts and throats.

2. Black hen B 2, which had received an ovary from a white pullet and was mated with a white cock, produced nine white chicks and eleven white ones having black spots on heads, wings, or backs.

3. Black hen B 3, which had received an ovary from a white pullet and was mated with a black cock, produced four ordinary black chicks (with light under surfaces) and two chicks described as being "black with white legs."

4. The control white hen mated with a white cock produced eighteen white chicks.

5. White hen W 2, which had received an ovary from a black pullet and was mated with a white cock, produced three white chicks, one chick white with black spots, and one ordinary black.

6. White hen W 3, which had received an ovary from a black pullet and was mated with a black cock, produced twelve white chicks spotted with white on head, wings, or back.

The conclusions to be drawn from these observations will be discussed elsewhere in this paper.

Magnus (1907) transferred the ovaries from an albino to a black rabbit with apparent success. The black rabbit was mated with an albino male five months after the operation and a month later bore two young, one black and the other an albino. Two months later she died pregnant, and in the uterus were found two dark-colored embryos and five light-red ones supposed to be albinos. No ovary was found on one side of the body, but on the other side was a well-developed and functional ovary bearing corpora lutea. Magnus supposes that all the embryos produced were derived from eggs liberated by the transplanted ovary, but in view of our own experience we are inclined to question this interpretation.

Ten other rabbits similarly grafted by Magnus produced no young, though three of them gave indications by their sexual activity that they contained living ovarian tissue.

Among other authors who have reported successful results with homoplastic grafting in animals may be mentioned: Schaus, Basso, Mauclaire, McCone, and Lukaschewitsch.

McCone gives the case of the birth of five well-formed offspring in a rabbit from the grafted ovary of another rabbit, but the evidence that the functioning ovary was an introduced ovary is far from complete. Pregnancy took place four months after complete castration and transplantation from another member of the same species. The other authors report no young.

At variance with these results are the work of the following: Marchese, Herlitzka, Marshall and Jolly, Burckhardt, and Preobrazhenski, in whose work degeneration of the implanted foreign ovary was complete or nearly so after short intervals.

The work on heteroplastic ovarian grafting is small in amount, and the evidence, taken as a whole, makes it safe to say that persistence of normal tissue after a few days or weeks is very rare.

Bucura (1907) reports the most favorable results. He transplanted ovaries of guinea-pigs into castrated female rabbits. In the first of his three cases he found after eight weeks the uterus atrophied, but some evidence of ovarian tissue without follicles. Another rabbit (similarly treated) was killed after fifty-one days. In this animal there was no genital atrophy, and one of the guinea-pig ovaries showed some normal follicles and a well-developed corpus luteum. The other was completely atrophied. In the third case, after seventy-seven days, one of the grafted guinea-pig ovaries was completely resorbed, the other nearly so; while atrophy was advanced in the rabbit uterus. The author thinks that heteroplastic transplantation is possible.

Basso (1905) reports negative results in grafting ovaries between guinea-pigs and rabbits.

McCone (1899) cites a case of persistence of the ovaries of a bitch in a female rabbit for three and a half months with prevention of genital atrophy in the rabbit. He gives an illustration of this ovarian tissue.

Lukaschewitsch (1901) believes that it is possible to transplant ovaries from carnivorous to herbivorous animals and *vice versa*, and that genital atrophy may be prevented. He obtained no young from animals so operated upon, and does not describe his operations in detail.

3. REVIEW OF THE LITERATURE ON OVARIAN GRAFTING IN MAN.

It is not surprising when we turn to the clinical experiments to find that autografts in women are by no means always successful. The material to be grafted has been adult, and the organ often affected by tumor growth or cystic and atrophic changes. Small wedges of the organ have been used.

Many authors have advocated conservative surgery in order to avoid the bad effects of precipitate menapause, but too often cases of this sort have been reported as successful when the after history was incomplete. It is safe to say that autografting of ovarian tissue in women has not given nearly such uniform results as in animals under experimental conditions.

Menstruation has been said to persist in as many as 20 per cent of all cases of ovariotomy. Furthermore, there are numerous cases reported of pregnancy after complete ovariotomy. Doran (1902) cites three such cases. In one of these, observed by himself, a definite focus of extra-ovarian tissue was found at a second operation in the ovarian ligament.

Dauber (1905) shows how difficult it is, especially in disease, to assert with confidence that all ovarian tissue has been removed.

Dudley (1900) cites several cases of pregnancy after ovariotomy.

Croom (1905) reports a case of homoplastic ovarian graft, with four years later the birth of a living child. Obviously it is impossible to verify such cases as this, and they must always remain open to doubt unless the child in question shows some strongly inherited and dominant characteristic which was possessed by the individual who supplied the graft, but not by the foster-mother or by the father. Such conditions are not likely often to be realized in clinical experience, hence our principal reliance for determination of the theoretical questions involved must be animal experimentation.

Morris (1906), who had done much successful clinical work on autografting, besides some early experimental work, reported the birth of a living child in a woman into whom, after careful castration with the Tuffier angiotribe, he had grafted wedges of ovary from another woman. The castrated ovaries of the woman who was grafted were cirrhotic, and no formed Graafian vesicles were found. It was assumed that the introduced ovarian tissue was responsible for the result reported; but Lucas-Championniére, criticizing this case, has expressed the view that it can be given another interpretation. He cites as an example a patient of his own, in whom, after the most careful castration with the angiotribe for a case of dysmenorrhea, the symptoms still persisted. A second operation on this patient, over a year later, revealed an important ovarian fragment at the uterine horn.

Glass (1899) gives a convincing case of homoplastic operation in a woman who two years before had had ovariotomy performed. She was suffering from all the symptoms of an artificial menapause, but a graft from another woman relieved her. The relief appears to have been permanent. No children are reported.

Mauclaire (1900) collected thirteen cases of homoplastic operation in women. In six of these cases menstruation returned, and in two cases it was resumed temporarily. The persistence and functioning of the introduced tissue is, however, in no case established beyond question. On the whole, Mauclaire regards ovarian transplantation as of doubtful clinical value.

Sauvé (1910) sums up in a very clear and direct way all the surgical evidence at hand. His statistics show that return of menstruation is no more common after ovarian grafting than after ovarian castration. There are, he thinks, only two cases of successful homoplastic grafting in women. The first case is that reported by Morris, in which a woman with infantile ovaries became normal and had regular menstruation after the transfer of ovarian tissue from another woman; and the second case is that of Pankow, which is supported by definite histological evidence after a period of three years.

4. NEW OBSERVATIONS ON OVARIAN GRAFTING.

In this paper we shall report upon seventy-four cases of homoplastic ovarian grafting in guinea-pigs and seventeen similar cases in rabbits. The purpose of the experiments represented by these cases has been stated in the introduction.

The operative technique in the guinea-pig was as follows: The hair of the female to be grafted was removed from each side at the costal border. She was then anesthetized and was castrated through an incision on each side, about an inch long, at the costal border and usually a little ventral to the ovarian site. The ovary was drawn into the field with blunt forceps and lifted with a small, double eye-hook, after having been carefully shelled out from the tube and mesentery. The organ was then cut away, care being taken not to damage the tube. The animal from which grafts were to be taken was then quickly killed and its ovaries removed, but with a small bit of mesentery attached. The ovaries themselves did not come into contact with any instrument. They were attached by means of very fine silk (OO untwisted into thirds) and a beader's needle, to various sites in the peritoneal cavity. Some even were merely dropped into the abdominal cavity. The muscles and skin were closed separately. At first two operations were made for each animal, but this was soon found to be unnecessary. The mortality was very slight. Out of the seventy-four cases six died as the immediate result of the operation; four of these were cases in which a ventral incision was tried.

To sum up the result of the entire series, only one* grafted animal had young from her grafted tissue; grafted ovaries functioned in six other cases, but did not produce young. Ten animals regenerated their own ovaries, and three of these had young. Forty-two showed post-mortem complete atrophy of the genital tract and absence of ovarian tissue. The remainder comprises fifteen cases in which results were not fully determined.

It is thought worth while to give an account of all the groups, because, though only one* case, Group I, bears on the problem before us, the series may have some physiological significance on account of its very considerable size, and may throw a side-light upon what criteria are necessary in such work.

GROUP I.

This group includes homoplastic transplantation of an ovary resulting in the birth of young derived from grafted tissue. On January 6, 1909, the left ovary was removed from an albino guinea-pig, No. 27 (fig. 2, pl. 1), then about 5 months old, and the ovary of a pure black guinea-pig (compare fig. 1, pl. 1), about a month, old was fastened near the tip of the uterine horn, distant a centimeter or more from the site of the ovary removed. One week later, January 13, a second operation was performed, in which the right ovary of the albino was removed, and as a graft was introduced the

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A second case, from a new series of experiments, has just been observed (January, 1911); see Postscript, p. 10.

ovary of a second young black guinea-pig, of like age with the first but of different ancestry. After the albino had fully recovered from the second operation, she was placed with an albino male, No. 654 (fig. 3, pl. 1) with which she remained until her death about a year later.

On the 23d of July, 198 days after the first operation, she gave birth to two female young. One was black but bore a few red hairs. A photograph of this animal (No. 1970) at the age of three or four months is shown in fig. 6, pl. 2. The other young one (No. 1969) was likewise black, but had some red upon it, and its right forefoot was white. (See fig. 5, pl. 2.)

On October 15 the grafted albino bore a third black young one, a male which, like those previously born, had a few red hairs interspersed with the black. A photograph of this animal is shown in fig. 4, pl. 2.

On January 11, 1910, the grafted albino was observed to be pregnant for the third time, and this time she was very large. Unfortunately, on February 2, she died of pneumonia with three full-grown male young in utero. The skins of these animals were saved and a photograph of them is shown in figs. 7–9, pl. 2. Like the other three young they were black, but with a few red hairs among the black ones. They bore no white hairs.

An autopsy made one hour after the death of the mother showed on the left side a distinct ovarian mass about a centimeter from the coiled part of the oviduct; that is, approximately in the position where the graft from the pure black guinea-pig was fastened at the first operation. On the right side the mesentery of the oviduct was adherent to the body-wall where an incision had been made at the second operation, and a small amount of tissue, regarded as possibly ovarian, was there observed. Both this and the ovarian tissue from the left side were preserved in Gilson's fluid for sectioning and microscopic examination. The tissue from the left side was found to contain numerous large egg-follicles, some already well advanced containing a lymph space; in addition a number of corpora lutea were observed. On the right side was found a small amount of undoubted ovarian tissue, with one well-advanced egg-follicle, but the whole apparently was strongly encapsulated, so that no eggs could be discharged even if they came to maturity.

It is interesting to note that both grafts persisted, though taken from different animals and transferred at different times. This result suggests a possible susceptibility on the part of the animal grafted.

Female 1970 (fig. 6, pl. 2) daughter of the grafted albino, was mated with the albino male, her father (fig. 3, pl. 1), and bore three young, two of which were albinos and one black with some red hairs. If female 1970 had been the daughter of a pure-black mother, instead of a grafted albino, we should have expected her to produce an equality of black and of albino young. The observed result was the nearest possible numerical agreement with this expectation.

A control mating of the albino male, 654, was made with a female of pure black stock. As a result there were produced two litters of young,

including five individuals, all black, but with red hairs interspersed. This result shows that the red hairs found on the six young of the grafted albino were due, not to foster-mother influence of the grafted albino, but to influence of the male parent. The young of the grafted mother were exactly such in color as the black guinea-pig which furnished the graft herself might have been expected to bear had she been mated with male 654 instead of being sacrificed to furnish the graft. The white foot borne by one of the young forms no exception to this statement. Spotting characterized the race of guinea-pigs from which the father came. He was himself born in a litter which contained spotted young, whereas neither the pure-bred black race that furnished the graft nor the albino race that received it was characterized by spotting.

Inasmuch as the offspring of albino parents are invariably albinos, it is certain that the six pigmented offspring of the grafted female were all derived from ova furnished by the introduced ovarian tissue taken from a black guinea-pig. This tissue was introduced while the contained ova were still quite immature, and it persisted in its new environment for nearly a year before the eggs were liberated which produced the last litter of three young. These young, like the earlier litters, gave no indication of foster-mother influence in their coloration.

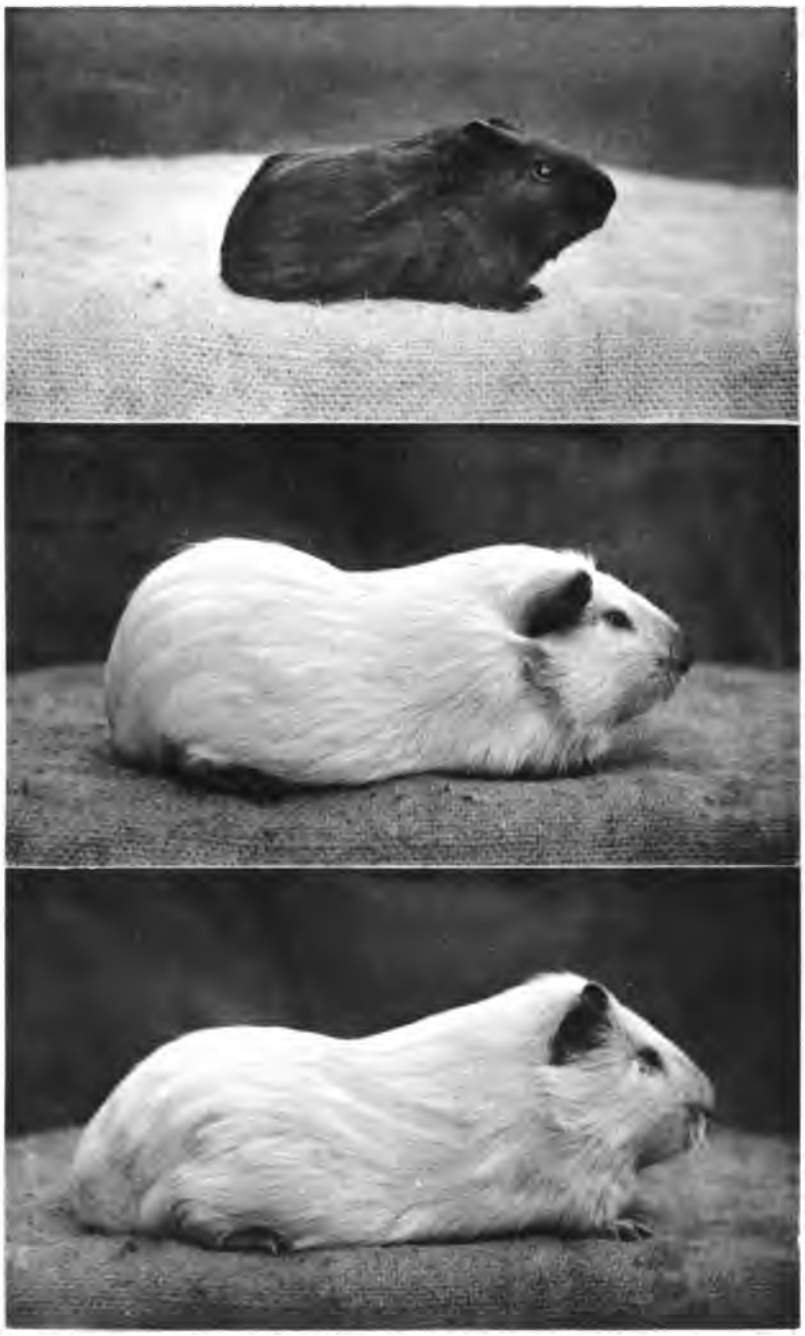
The conclusion is forced upon us that the egg-cell during its growth does not change in germinal constitution. Its growth is like the growth of a parasite or of a wholly independent organism: what it takes up serves as food; this is not incorporated merely in the growing organism; it is *made over* into the same kind of living substance as composes the assimilating organism.

POSTSCRIPT.

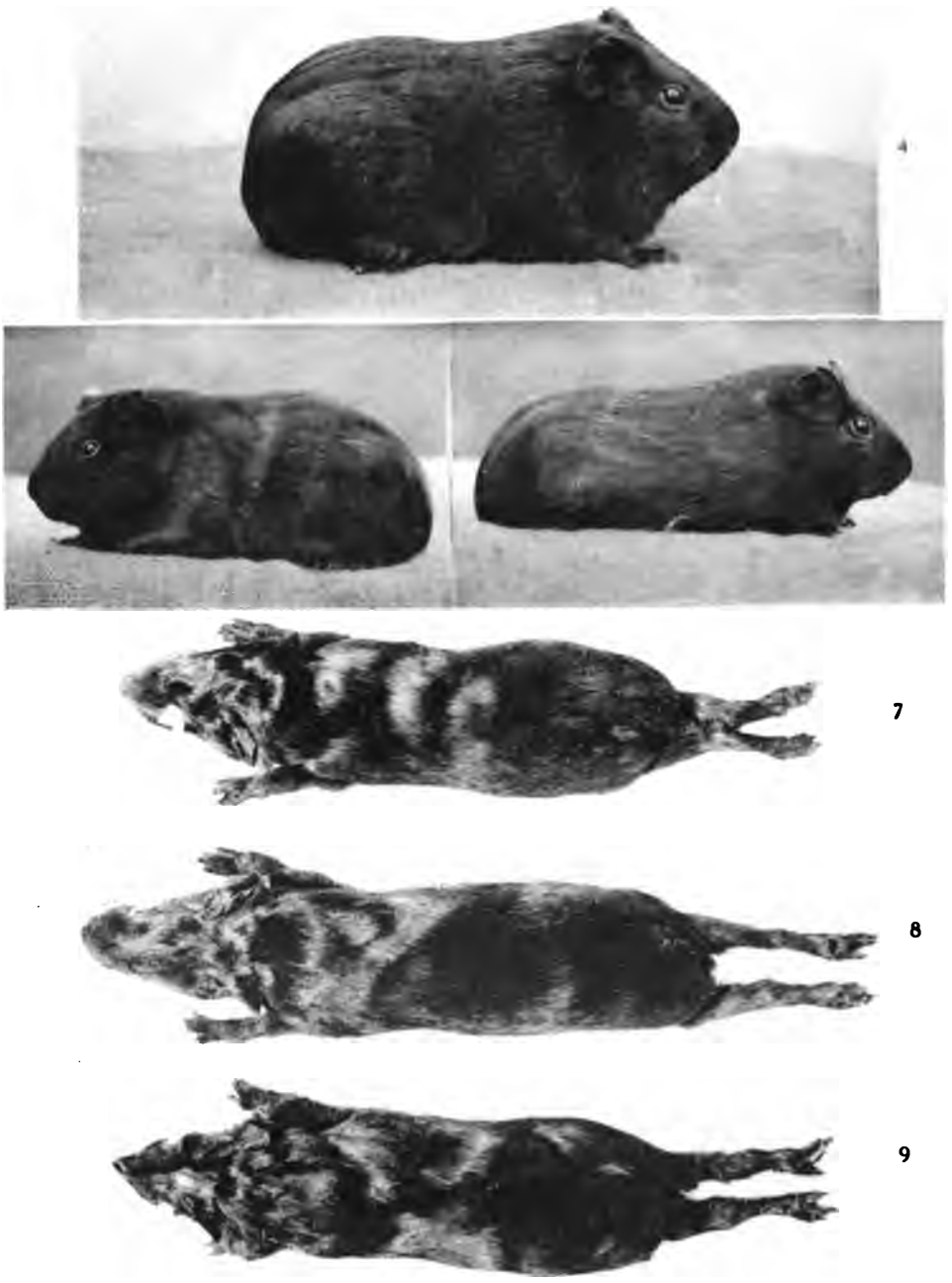
While this paper was in press a second case, from a new series of experiments, belonging in Group I came to light.

An albino female, No. 2475, born on the 22d of March, 1910, at 75 days of age was operated upon, being doubly castrated and receiving the ovaries from a half-sister, No. 2507, a brown-eyed cream animal, 17 days younger than herself. She was then mated with an albino male, her half-brother, No. 2402. On October 20, 136 days after the operation, she gave birth to an albino young one. We considered this an indication that the castration had been imperfect, but allowed the animal to breed again. Much to our surprise, 73 days later, on January 1, 1911, she gave birth to two young, one of which was a brown-eyed cream like the animal which furnished the graft. The other young was an albino.

The albino young borne by this grafted animal can not properly be regarded as evidence of somatic influence on the introduced graft. For albinism occurred as a recessive character in the particular brown-eyed cream stock used, as is evident from the fact already stated that the colored animal which furnished the graft and the albino which received it were half-sisters. The character of the young obtained and their numerical proportions are exactly such as the colored animal would herself have been expected to give, had she not been sacrificed to furnish the grafts, but had been mated with the albino male.



1. A Young Black Guinea-Pig, about three weeks old. Ovaries taken from two animals like this were transplanted into the albino shown in Fig. 2.
2. An Albino Female Guinea-Pig, No. 27. Its ovaries were removed and in their place were introduced ovaries from two young black guinea-pigs. Compare Fig. 1.
3. An Albino Male Guinea-Pig, No. 654, with which was mated the albino shown in Fig. 2.



4, 5, and 6. A Group of Three Young, produced by the pair of albinos shown in Figs. 2 and 3. 4, is female 1969, 5, is female 1970, 6, is the male born on October 15. All were about half grown when photographed.

7, 8, and 9. Skins of three young found in utero at the autopsy of female No. 27, Fig. 2. The sire of these young also was male No. 654, Fig. 3.

GROUP II.

In this group are included grafted females which bore no young but in which, post mortem, there was found ovarian tissue remaining from the grafts. Of these there are six cases. It is not possible in each case to say why no young resulted. In five cases the egg-follicles were well developed, but there is some evidence of the encapsulation of the graft. The age of these animals varies from three months to a year. Their own ovaries had been, of course, always first removed before the transplantation was made.

Guinea-pig No. 40, age four weeks, was grafted from an animal three weeks old. One ovary was sutured to the body-wall near the incision on the right side. The other was dropped into the abdominal cavity. Nine months later the animal was killed and found to have a healthy uterus. At the right body-wall was a suspicious nodule, which was preserved for reference. Tissue at the right ovarian site was also saved. Microscopic examination showed the specimen from the body-wall to contain much ovarian tissue and numerous Graafian follicles. The right ovarian site showed no ovarian tissue.

Guinea-pig No. 61, age one year, was grafted from a younger animal, exact age not stated. The grafts were placed, one loose in the abdominal cavity, and one stitched to the muscle wall on the right side near the incision. Five and a half months later the animal was killed. Her uterus was found large and healthy, and on the right body-wall was a vesiculated mass 20 mm. long, containing what appeared to be the transplanted oviduct in a state of hydrosalpinx, together with a small, pink, hard mass at one end. Later this mass was found to be ovarian. One young egg-cell in its follicle was seen. A specimen of the right ovarian site showed no ovarian tissue.

Guinea-pig No. 21, age six months, was grafted from an animal one month old. The grafts were attached one on each side, to mesentery close to the horn of the uterus. Four and a third months later the animal was examined, and both grafted ovaries were found, easily identified by their position. The uterus was in the stage of heat, and microscopic examination showed both ovaries to contain Graafian follicles. One egg was found undergoing a maturation division. There was no ovarian tissue at either original ovarian site.

Guinea-pig No. 53, eight months old, was grafted from an animal one month of age; the operation was a double one, that on the right side being done one week after that on the left side. The ovaries were attached, one on each side, to the mesentery at the uterine horn. Four months later the animal showed a large uterus in the stage of heat, and on the right side was found the grafted ovary, small and pale. It contained many large Graafian follicles. There was no other ovarian tissue found anywhere.

Guinea-pig No. 47, three months old, was grafted from an animal three weeks old. Both the ovaries were dropped into the body-cavity through the

left incision. Ten and a half months later the animal was killed. The uterus was well nourished. There was no tissue at the sites of the original ovaries, but attached to the border of the greater omentum was a typical ovarian mass which showed microscopically plenty of ovarian tissue and at least one large ovarian follicle. This case is interesting as showing that presence of ovarian tissue in immediate contact with the genital ducts is not necessary to prevent atrophy of those ducts. An ovary attached to the digestive tract (or doubtless anywhere else in the body) serves equally well to prevent genital atrophy. Some substance liberated by the ovary and circulating in the blood is doubtless the active agent in maintaining the full development of the uterus and the recurrence of rut.

In guinea-pig No. 73, one year old, the grafts were placed, one attached to the right body-wall, the other merely dropped into the abdominal cavity. Nine and a half months later the animal was killed. There was little or no genital atrophy, and at the site of the graft on the body-wall a small mass was found which was identified by microscopic examination as the oviduct of the graft, with a small amount of degenerating ovarian tissue, without follicles. There was no sign of regenerated tissue at the ovarian sites.

The above six cases show that normal ovarian tissue persisted in its new environment for periods from four months to ten and a half months. They demonstrate that the position of a homoplastic graft may be varied to any place on the peritoneal surface; and that the grafted ovary may attach and nourish itself without the aid of sutures.

The failure of these foreign ovaries to bring about pregnancy inclines one to the idea that the ovum when mature and liberated may be more subject to resorption during its migration to the uterus than the more primitive tissue from which it was discharged. It is also well to remember that Graafian follicles have been found to be less in number and larger in size in grafted tissue than in normal tissue (Carmichael, Sauvé). Further, in the case of an ovary removed from its normal position, the chances are necessarily greatly diminished that the egg when liberated will find its way into the oviduct. In an ovarian operation there is also great danger that the open end of the oviduct will become permanently closed in consequence of injury. On the whole it is not surprising that foreign ovaries often persist without bringing about pregnancy.

Group III.

This group includes ten cases in which, in spite of a supposed perfect castration, the animal regenerated ovarian tissue at the original site. Three of these animals bore young. In all ten cases regenerated ovaries were found post mortem by inspection and microscopic examination. The color-character of the young indicated that they had probably come from regenerated tissue, and the autopsy in all cases confirmed this view.

The three cases in which young were born are as follows:

Guinea-pig No. 77, blue, age five months, underwent a double ovarian operation in which grafts were taken from a pale cinnamon agouti guinea-pig, age one week. Both grafts were placed on the right side at the uterine horn. Six months later she bore by a white male, No. 654 (fig. 3, pl. 1), a blue female young one. She was killed eight months after the operation and ovarian tissue was demonstrated at the right ovarian site, while the graft had completely disappeared.

Guinea-pig No 24, an albino, age four weeks, was grafted from a black animal one day old. One graft was stitched to the body-wall on each side. Two and a half months later she was noted pregnant, and at three months she had, by male 1078 (a brown-eyed yellow), two young, both black-eyed yellow. Her post mortem two days later showed indications of ovarian tissue at both ovarian sites, but the microscopic examination showed that ovarian tissue was confined to the right ovarian site. There was nothing left of either graft.

Guinea-pig No. 1907, black, age three weeks, whose parents were one black, the other cinnamon agouti marked with cream, was grafted with ovaries from a cinnamon agouti, age five days. One graft was dropped into the abdominal cavity, and one was attached to the body-wall on the right side near the incision. Five months later she bore, when mated to male No. 654, fig. 3, a young black male with red brindling. The post-mortem showed at the right body-wall a pinkish mass 1.5 mm. in diameter, which was preserved. Both ovarian sites were also saved for microscopic examination. The peritoneal cavity was negative. The microscope showed that the mass on the body-wall consisted of much fibrous and connective tissue penetrated by blood-vessels, the remains of the degenerated graft. The right ovarian site showed normal ovarian tissue with Graafian follicles present.

The other seven cases, in which no young were produced, but in which the presence of regenerated ovarian tissue was demonstrated by the microscope, need not be given in detail. A well-developed condition of the uterus was observed in all.

Group IV.

In this group are included those cases, forty-three in all, which showed, by complete atrophy of the genital tract, absence of all ovarian tissue. Careful microscopic inspection was made in all cases, but nothing resembling ovarian tissue was in any case observed. Four of these animals died from two to nine months after the operation; the remainder were killed at periods ranging from four months to one year afterward. The average age of the lot at the time of the operation was eight weeks. The individual age of the transplanted ovaries varied from one day to two months, while the average age was three weeks.

GROUP V.

In this group are placed fifteen cases in which the grafted animals died or were lost without any autopsy being made upon them. Only six died as the immediate result of the operation, four of the six being cases in which a ventral route to the ovaries was attempted.

The results obtained by us from ovarian grafting in rabbits are largely negative. Seventeen animals have been operated upon.

One rabbit, in which an autograft was made onto the uterine horn on each side, gave birth four months later to two young. Nine months later a second operation showed both these autografts functioning; they were removed and ovaries from another rabbit were placed in their stead; atrophy of the external genitalia soon resulted and the animal was a few months later discarded.*

Of the other homoplastic cases, two showed regenerated tissue at the original ovarian site, one of these having had young. Of the remainder, six died or were lost, while three showed complete atrophy of the external genitalia and three more are still alive, manifesting normal sexual instincts but having borne as yet no young.

On the whole the results obtained from rabbits are very similar to those obtained from guinea-pigs. Autografting is evidently not difficult, and may be followed by the birth of young. In heteroplastic grafting, regeneration of ovarian tissue at the original site is a commoner event than growth of the introduced tissue, the latter result not having been certainly obtained in our experiments with rabbits, though the former has been obtained in two cases.

5. CRITICAL EXAMINATION OF THE OBSERVATIONS OF GUTHRIE AND MAGNUS ON SUPPOSED FOSTER-MOTHER INFLUENCE.

A cursory reading of Guthrie's evidence may well lead one to accept without question his conclusions: (1) that in his fowls the transplanted ovaries have functioned, and (2) that the foster-mother has influenced the character of the young; but critical analysis of the evidence fails to substantiate either conclusion. Since the ovaries exchanged were transplanted to the original ovarian site, it would have been impossible by autopsy to ascertain whether the ovary present was transplanted or regenerated tissue. But regeneration is a much commoner result of such operations than successful transplantation, as our results with guinea-pigs show; and in the absence of other evidence the interpretation of the functioning ovary as a regenerated ovary should be preferred. The only other evidence we have is that derived from the character of the offspring.

*Simple inspection of the external genitalia appears to be sufficient in rabbits to show whether or not the uterus is functioning normally.

Since in Guthrie's experiments the character of the germ-cells furnished by the mother is in question, we may conveniently group the experiments according to the character of the male used.

Series I. Rooster Black.

Black hen B 1, control, mated with the black rooster, had 13 black chicks with "grayish-yellow breasts and throats, and frequently the under surfaces of the tips of the wings were light colored as well, but the plumage on the entire dorsal surface was *always* solid black."

Black hen B 3 and white hen W 3 had their ovaries exchanged and were later bred to "the black rooster." Black hen B 3 produced six black chicks, four apparently normal, but two "with white legs." We are not told whether this whiteness was of down or final plumage, but the statement is added: "In regard to the chicks from this hen described as ordinary black, some doubt exists as to whether the ventral light-colored area described for normal black chicks was not lighter and greater in extent in all cases than in the normal chicks." Since this ventral lightness is of down only, not of later plumage, we are led to infer that the description of the abnormal black chicks was also based on the down colors. And since, further, white chicks are not *white* in the down, but blacks chicks are *light* below in the down, it seems that Guthrie's real meaning is that the two abnormal chicks had *light* legs (not white legs) in the down. But this does not indicate whether the adult plumage in that region was to be black or white. If this reasoning is correct, then the only difference between these two chicks and ordinary black chicks is that they perhaps had more extensive light ventral areas in the down plumage, the light area extending onto the legs. This has no relation whatever to the plumage condition of the white race from which the graft was taken and can not fairly be assigned to that source.

If the six chicks were produced from regenerated ovary (*i. e.*, from germ-cells of the black hen not removed at the operation) we should expect them all to be black, as they were. If they were produced from transplanted ovarian tissue without foster-mother influence, we should expect them all to be white, which they were not. For the white of Leghorn fowls, according to Bateson and Davenport, behaves as a dominant character in crosses with black, cross-breds being white or white spotted sparingly with black. Unless, therefore, we grant that these six chicks came from regenerated ovarian tissue of the black mother herself, we are forced to assume that the ovarian tissue transplanted from the white hen completely changed its character from white to black within the black hen. It is a hypothesis at least equally plausible to suppose that no cells were changed in character, but a few were overlooked in the castration.

White hen W 3, having received the ovary from black hen B 3, was also mated with "the black rooster." She produced twelve "white chicks with black spots on the dorsal surface of the head, neck, wings, back, or on the

tail." This is such a result as we should expect had the hen not been operated upon at all. It is fully accounted for if we assume that the ova which produced these twelve chicks came from regenerated ovarian tissue of the white mother herself. On the other hand, if the transplanted ovary had produced the chicks without any foster-mother influence, the chicks should have been all black with light ventral surfaces, which they were not. Again the regeneration hypothesis presents far less difficulty than that of a successful transplantation.

Series II. Rooster White.

White hen W 1 control, mated with the white rooster, had eighteen chicks "pure white to light buff when hatched." White hen W 2 and black hen B 2 had their ovaries exchanged, and were later bred to the white rooster.

Black hen B 2 produced nine white chicks and 11 white spotted sparingly with black. Such a result as this is the usual consequence of a cross between black fowls and white ones; that is, it is what might have been expected had the black hen never been operated upon. And, on the other hand, if the functional ova came from the transplanted ovary, the expectation would not be materially different, though the *breeding capacity* of the young would be different in the two cases. Unfortunately this was not tested.

The white hen W 2, which had received the black ovary from B 2, produced five chicks. Three were white, one white spotted with black, and one black. A white hen not operated upon might be capable of producing all three sorts when mated with a white rooster of a similar character; that is, both parents might be Mendelian heterozygotes bearing black as a recessive character, in which case one-quarter of the young should be black. Now, the numerical result is not at variance with such an interpretation. But if the *same* white rooster was used in this mating as with B 2, it is surprising that no black young were produced by B 2. Guthrie does not expressly state that the same rooster was used in both cases, but we assume this to have been the case from his use of the expression "*the* white rooster." If different males were used in the two cases, one may well have been homozygous, the other heterozygous. In that case the black chick as well as the white and the spotted ones may have come from regenerated, not from transplanted tissue. But if the same white rooster was used in both cases, it is still equally difficult to account for the black chick as a product of a transplanted germ-cell or of regenerated ovarian tissue of the white mother. For unless the white male was heterozygous in black we should expect *no black young* to be produced even were every egg which he fertilized produced by a pure-black hen, instead of by a white hen possibly carrying borrowed black germ-cells.

Control matings of the white rooster with normal black hens, or of the black chick when it became adult, would have cleared up the case, but no control crosses were made by Guthrie. The control mating of "the white rooster" with a normal white hen W 1 was not a sufficient control of his

breeding capacity. It does not prove him to have been homozygous in white. If W 1 was homozygous, only white chicks would be expected, whatever the character of the white rooster.

We have now examined in detail the two pairs of transplantation experiments which form the entire basis of Guthrie'e assumption that the foster-mother may in a case of homoplastic transplantation affect the character of the offspring. In no one of the four cases does the hypothesis that the transplanted ovary functioned offer less difficulty than the hypothesis that only regenerated ovarian tissue functioned. In two of the four cases the advantage is overwhelmingly with the latter hypothesis. That ovarian tissue might easily be left behind in exchanging ovaries between two animals Guthrie frankly admits in a recent paper (1910), citing experiences of his own to show just how this might come about. He still maintains, however, his belief in foster-mother influence, because, we believe, of a failure to grasp fully the laws of inheritance of the character which he used as a criterion. A similar failure is shown by the comment which he makes in Science (1909) upon the case which we have more fully described in this paper (Group I). Referring to our failure to detect foster-mother influence in the young borne by female 27, he says: "Had the operated pig been bred to a male of the same strain as the pig from which the engrafted ovary was obtained * * * characteristics in the offspring indicative of such influence might have been obtained." Now, suppose the operated pig had been so bred, what result might have been expected? Exactly that which was obtained from the mating with an albino, only we should have been left in uncertainty, precisely as in Guthrie's own experiments, as to whether the pigmentation of the offspring was due to maternal or paternal influence. By the mating with an *albino* male all such uncertainty was eliminated, since it was rendered sure that if the offspring were pigmented they could have derived this character from no other source than the transplanted ovary of the little black guinea-pig.

Our case does not prove that foster-mother influence is impossible. No such claim is made for it. But our observations do show that evidence such as Guthrie has presented is wholly without value in establishing foster-mother influence, and that in one specific case, the first critical case on record, we believe, no foster-mother influence is detectable.

In view of our own experiments on guinea-pigs, in which the source of the tissue which liberated the ova is fully established and in which no foster-mother influence is detectable, we may fairly ask that the experiments with fowls be repeated on adequately controlled material before we accept the interpretation which Guthrie has given to his results.*

*While this paper was in press our attention was called to the fact that Dr. C. B. Davenport (1910, Proc. Soc. Exp. Biol. Med., vol. VII, p. 168) had indeed repeated Guthrie's experiment with fowls, on material adequately controlled and of known pedigree. The result was that in every case castration was incomplete and ovarian regeneration occurred, leading to the production of young showing no influence of the introduced graft.

The work of Magnus demands no extended discussion. Into a black rabbit were introduced the ovaries of a white one. The grafted black rabbit was then mated with a white male rabbit. Two litters of young resulted which consisted in part of black animals and in part of white ones. There is no question of an intermediate or modified character in the young, such as Guthrie would have us believe occurred in his experiments. If any foster-mother influence was exerted at all, it was such as changed the character of germ-cells completely from the albino to the fully pigmented condition. But this supposed influence manifested itself in part only of the germ-cells subjected to the new somatic environment; the others were unaffected.

An alternative interpretation is plainly possible. The ovary observed at the autopsy of the grafted black rabbit may have been regenerated from fragments of ovarian tissue not removed by castration. We do not understand that there was anything in the observed position of the organ decisive either for or against such an interpretation.

It is known from studies of Hurst and of Castle that the albino condition in rabbits is a Mendelian recessive to black. The grafted black rabbit may have been a heterozygous black, containing albinism as a recessive character. In that case, if the black rabbit had not been operated upon, but had been mated with an albino male, we should have expected that young would be produced half of which were black, half albinos. This is substantially the result observed. Accordingly, so far as the character of the young is concerned, there is no reason to think that they were produced from introduced ovarian tissue rather than from regenerated ovarian tissue of the black rabbit herself.

But if we adopt the interpretation that the observed ovary was really an introduced ovary, we encounter this difficulty. If introduced egg-cells through somatic influences were changed in character so that they produced black young instead of white ones, why were not *all* the egg-cells so affected? Were they not all subject to the same somatic influences? On the whole, therefore, we meet fewer difficulties by interpreting the observed ovary as a regenerated ovary rather than as a transplanted ovary.

6. A REVIEW OF THE LITERATURE ON GRAFTING OF TESTICLES.

The work on testicle grafting is less voluminous than that on ovarian grafting. In a general way some of the more uncritical of the early investigators reported results of a positive nature; but later work along the same lines gives results of an entirely different character.

Berthold in 1849 interchanged testicles between two cocks after castrating both animals. The organs, one in each animal, were not sutured. Five months later they were found grown against the colon in both cases, and full of active spermatozoa. The cocks retained their sexual instincts.

Mantigazza (1860), experimenting on frogs, found living testicular tissue in grafted animals after a period of 70 days. He thought that the tissue preserved its structure. Bizzozero (1868) confirmed these results.

Cheveau (1890) in a brief note described autoplastic testicular transplantation, apparently of adult tissue, in sheep. He obtained nourishment of the exterior of the testicle, but concludes that the operation is futile if the testicle is really detached from its own blood-supply.

A. Lode (1891) reported the results of experiments on cocks which were castrated, their organs being transplanted into subcutaneous connective tissue. One cock eight months after operation revealed a tumor of bean size under the skin. This appeared vascularized and contained living spermatozoa. Another cock, in which, after being caponized by a professional caponizer, the testicles were thrown back into the body cavity, showed a bean-sized testis attached to the peritoneum at the site of the old wound, containing living spermatozoa. There were also numerous spermatozoa contained in little testicles in various parts of the body cavity, which were regenerated bits of the testicles, crushed in the process of caponizing. He states that he always found testes remaining in capons, no matter how carefully castrated. This work therefore would allow one to make a somewhat different interpretation of Berthold's work than he himself made: the supposedly transplanted testicles may have been regenerated from fragments never removed.

Hanau (1897), also working on fowls, found difficulty in completely castrating cocks, and showed that a small amount of the testicle, if left behind, forms little capsules, containing sperm and embedded in connective tissue. Testicles transplanted into hens were encapsulated and resorbed.

In 1898 Goebell, questioning the results of Berthold, operated on guinea-pigs and found that transplanted testes in the body-cavity became necrotic after two days, except the most superficial layers, which showed mitoses.

Herlitzka (1899), in a very critical paper, reports the results of the transplantation of testicles in tritons. In all, 32 animals were used, 20 females and 12 males. These experiments were in part planned to test the theory of Ribbert (1898), who, working on a large series of rabbits, found degeneration to take place, or more correctly a process of regression to a more primitive type of cell, both when bits of tissue were placed in lymph nodules and when whole testicles, autoplastic or homoplastic, were fixed by a peritoneal suture. Ribbert found that the epithelium of the efferent canals resisted longer than the epithelium with specific function. He concluded that transplantation of the testicles was not possible because there is concerned a gland which discharges its secretion externally.

To test this last theory Herlitzka used tritons both in winter stages and in summer stages. The testicles were transferred from one animal to another, and also to females ; and in some males one of the animal's own testicles was left as a control. His results in brief were degeneration of the transplanted tissue under all conditions in from ten to fifty-two days, usually about a month. The state of the testicle, whether resting or active, made

no difference. The efferent parts of the tubules degenerated, though more slowly than the sperm-forming parts. The author is inclined to attribute non-success, in part at least, to the absence of trophic stimulation of the nervous system, though he admits his view is far from being proved.

Zalachas in 1907 gives the result of autoplastic transplantation in frogs, in which he found living sperm preserved after one month. He does not claim that this proves activity of the transplanted gland, for as a control he found (completely degenerated but containing active spermatozoa) a testicle which had been for one month in a sterile tube. Zalachas also transplanted testicles of very young dogs into older dogs, and in seven cases got almost complete degeneration at the end of one month.

Foges (1898) extended the result of Lode's work on caponizing cocks, without obtaining any very convincing results.

Foá (1901), in a very able paper, sums up the results of others, and then describes in detail his own work on dogs, autoplastic and homoplastic, which gave negative grafts after one month. The organs of three-day old dogs were used for the transfer. In another experiment a part of one of these testicles was carefully sutured by the tissues to another testicle, in which, though left *in situ*, a part of one end had been cut off. The cut faces of the two testicles were thus brought face to face. Complete degeneration took place both in the graft and in the remaining portion of the animal's own testicle.

Foá therefore combats the theory of the lack of efferent ducts suggested by Ribbert. His results are the same whether the testicle is embryonic or adult, whether whole organs or parts of organs are used. Foá gives weight to traumatism, brought about by the operator, as the essential cause of degeneration.

We believe that the results of Maximow (1899), in the study of the regenerative capacity of the testicle after artificial wounds, show the testicular tissue to be of a very delicate nature, easily unbalanced by slight mechanical disturbances. The work of Cevolotto (1909) goes far towards strengthening this view. This author made a careful study of the cell changes in autoplastic transplantation of bits of testicular tissue into the tissue of the ear. His results, in a word, show the harmful influence to the highly differentiated epithelial tissue, which last is quickly converted into embryonic tissue. Sertoli cells increase and epithelial giant cells appear.

With this idea in mind the results of Guthrie (1910) are indeed surprising, though it must be confessed that a review of the literature shows the cock to be a very likely subject for transplantation experiments. Guthrie reports normal testicular structure existing in grafts after four months. He hopes to produce artificial fertilization in hens from such testicular grafts in continuation of his experiments on the effect of the foster-soma on engrafted germ-cells.

7. NEW OBSERVATIONS ON GRAFTING OF TESTICLES.

In order thoroughly to satisfy ourselves whether germinal transplantation is possible when tissue of the male sexual gland is used as a graft, the following experiments were performed on rats before ovarian work was started. The operations were homoplastic and the technique simple. The animals used to supply the graft were new-born males or those only a few days old, while those into which the tissue was grafted were young adults of the same sex. No attempt was made to unite the efferent ducts, or to supply special arterial contact, the young testicle being simply dropped into the tunica vaginalis after its original contents had been carefully removed.

The rat was etherized, the scrotum sterilized and opened at its discal end by a small incision. The testicle was then drawn out, the tunic split, and the entire contents removed, leaving the end of the spermatic artery free. The other testicle, now quickly removed from the young animal supplying the graft, was carefully manipulated and placed within this tunica, sometimes with its own envelope intact and sometimes with one of its ends cut across. The tunica, now containing the young grafted testicle, was closed by fine silk sutures, the testicle replaced, and the scrotal wound also closed. There was considerable extravasation of blood from the spermatic artery in a few cases, distending the tunic and sometimes leaking through the sutures. The testicle of the other side was removed through a small incision in the scrotum and discarded, a suture being placed around its spermatic cord.

Thirty-three rats were thus operated upon. One died six days after the operation; the rest were killed at periods of from one month to two months after the operation. Four of them showed small abscesses in the operative region. The other twenty-eight gave no evidences of ever having been infected. In general, the appearance was very uniform. The epididymis was flabby, and in the region of the graft there was either a very small yellowish body or nothing but a bit of connective tissue surrounding the old stitches of the tunica. A number of the specimens were saved, but only one was considered to be promising enough to warrant sectioning. The microscopic examination was entirely negative so far as showing the presence of living testicular tissue was concerned, and so the experiments were discontinued.

It is evident from our experience, as from that of others, that testicular tissue is much more sensitive to injury, mechanical or otherwise, than is ovarian tissue.

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